Dynamic Assembly of the Receptor-G-Protein Signaling Complex

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a multidisciplinary approach combining computational and experiment work that leverages recent structural data, we present a model for the supramolecular structure of the rod cell disc membrane phototransduction machinery. We first show a comparison of multiple rhodopsin dimer interfaces in a model membrane using a coarse-grained molecular dynamics simulation approach accumulating more than a millisecond of simulation time. To characterize the preferred binding interface of a pair of rhodopsins, we determined the potentials of mean force as a function of the distance between two membrane-embedded receptors. The interfaces probed include helix 4 (H4), H4/H5, H5, H6 and H1/H8. The results show that the most stable rhodopsin dimer exists in a tail-to-tail conformation, with the interface comprising transmembrane H1 and H2 at the extracellular side and amphipathic H8 at the cytoplasmic one. The existence of the H1/H8 dimer was unambiguously corroborated by crosslinking experiments in which we identified CYS316 in H8 as the site of a chemical crosslink between two rhodopsins in native ROS disc membrane using proteolysis, CNBr cleavage, and high-resolution liquid-chromatography mass-spectroscopy (LC/MS). We then show how secondary interaction surfaces appear to stabilize extended ''lubricated'' rows of these dimers as seen earlier in atomic force microscopy studies. The synthesis of the new rhodopsin dimer orientation with the structures of the R*/Gt complex in two different orientations and 3-D densities of the complex obtained earlier by electron-microscopy argues for novel alternatives for the supramolecular organization of ROS membrane. The model we propose suggests the possibility for an efficient one-dimensional mechanism for Gt to search for active receptor (R*) even under low light conditions.

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Dynamic Assembly of the Receptor-G-Protein Signaling Complex

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The recent crystal structure[1] of the β_2 adrenergic receptor-Gs protein complex (β_2AR-Gs) marks an important milestone in a two decades long race to decipher one of the most important and widely utilized signal-transduction mechanisms in biology. However, it is unclear how the relatively drastic and unexpected conformational changes evolve from the initial encounter complex. The number of compositional and conformational microstates that evolve along the reaction pathway from the initial encounter of agonist-activated receptor with the GDP-bound heterotrimeric G-protein to the nucleotide-free agonist-receptor-G protein ternary complex is potentially very large.[2] Identification of these pathways is out of reach for crystallographic experiments, and we follow a complementary approach with large-scale molecular dynamics (MD) simulations of membrane-embedded, fully solvated metarhodopsin II alone and in complex with its cognate G-protein, transducin, in GDP-bound form. Here, we compare and contrast the relaxation of two different encounter complexes, one with an orientation similar to β_2AR-Gs, and the other 120° rotated. Interestingly, the two different pre-aligned encounter complexes yield induced-fit complexes that share a signature set of conformational changes involving highly conserved residues in the three allosterically coupled domains, the agonist-binding, G-protein-binding, and nucleotide-binding sites. How are these canonical and non-canonical binding modes possible for such a high fidelity signal-transduction system? Is it possible that Nature utilizes multiple, energetically similar states to relay the information encoded in the relatively small free energy of agonist binding across about ten nanometers distance to the nucleotide-binding pocket? It is tempting to speculate about the role of these energetically near-equivalent microstates when discussing the dominating entropic contribution to the binding free energy of agonists at the β adrenergic receptors.

[1]Rasmussen, et al. (2011) Nature 477, 549.
[2]Huber and Sakmar (2011) Trends. Pharmacol. Sci. 32, 410.

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Arrestin Allows All-Trans-Retinal to Enter the Ligand Binding Pocket of Phosphorylated Opsin

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Within the rod cell of the retina, absorption of light by the G-protein coupled receptor rhodopsin leads to the formation of the agonist all-trans-retinal directly within the ligand binding pocket of opsin and the eventual formation of the active species Metarhodopsin II (Meta II). Meta II signaling is terminated by phosphorylation and binding of arrestin, and Meta II eventually decays to opsin and free retinal via the spontaneous hydrolysis of the covalent retinal Schiff-base linkage. It has been known for some time that arrestin binds photo-decayed phosphorylated rhodopsin, as well as phosphorylated opsin (OpsP) supplied with exogenous all-trans-retinal. We find that all-trans-retinal can enter OpsP and form a Schiff-base in an arrestin-dependent fashion, thus forming a Meta II-like species. The agonist, arrestin, and the receptor exist in coupled equilibria, such that arrestin stabilizes an activated form of OpsP that allows all-trans-retinal to enter the binding pocket. This is the first direct observation of the reversible formation of a Meta II-like species from opsin and all-trans-retinal in the native membrane environment. As we recently reported for arrestin binding to Meta II (Sommer ME, Hofmann KP and Heck M (2011) JBC 286: 7359-69), we now find that one arrestin binds for every two OpsP receptors in the presence of excess all-trans-retinal. Furthermore, arrestin can induce the formation of the Meta II-like species in only half of the OpsP population. Likewise, regeneration of half the arrestin-bound OpsP population with 11-cis-retinal is blocked, supposedly because the binding pocket is already occupied. Regeneration of the remaining half of OpsP requires release of arrestin via the removal of all-trans-retinal by retinal dehydrogenase. Together these results suggest that arrestin serves to both terminate Meta II signaling and to regulate retinoid flow through opsin.

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An Activating Helix Switch at the Rhodopsin-Transducin Interface

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Extracellular signals prompt G protein-coupled receptors (GPCRs) to adopt an active conformation (R*) and to catalyze GDP/GTP exchange in the α-subunit of intracellular G proteins (Gαβγ). Kinetic analysis of transducin ($G_t\alpha\beta\gamma$) activation has shown that an intermediary $R^*\cdot G_t\alpha\beta\gamma\cdot GDP$ complex is formed which precedes GDP release and formation of the nucleotide-free $R^*\cdot G$ protein complex. Based on this reaction sequence we explore the dynamic interface between the proteins during formation of these complexes. We start from the R* conformation stabilized by a $G_t\alpha$ C-terminal peptide (GαCT) obtained from crystal structures of the GPCR opsin. Molecular modeling allows reconstruction of the fully elongated C-terminal α-helix of $G_t\alpha$ (α5) and shows how α5 can be docked to the open binding site of R*. Two modes of interaction are found. One of them - termed stable or S-interaction - matches the position of the GαCT peptide in the crystal structure and reproduces the hydrogen bridge networks between the C-terminal reverse turn of GαCT and conserved E(D)RY and $NPxxY(x)_{5,6}F$ regions of the GPCR. The alternative fit - termed intermediary or I-interaction - is distinguished by a tilt and significant clockwise rotation of α5 relative to the S-interaction. It shows different α5 contacts with the $NPxxY(x)_{5,6}F$ region and the second cytoplasmic loop of R*. From the two α5 interactions, we derive a 'helix switch' mechanism for the transition of $R^*\cdot G_t\alpha\beta\gamma\cdot GDP$ to the nucleotide-free $R^*\cdot G$ protein complex. It illustrates how α5 acts as a transmission rod to propagate the conformational change from the receptor-G protein interface to the nucleotide binding site. The results are discussed in light of the recent structure of the β_2 adrenergic receptor-Gs protein complex. A detailed mechanism of complex formation and GDP release is derived.

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Intermediates of the Channel Rhodopsin Photocycle

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Channelrhodopsins are sensory photoreceptors that mediate phototaxis in green algae (1, 2). Their function as ion channels renders them useful tools in the field of neuroscience. Due to the prolonged lifetime and light-sensitivity of the conducting state, mutantants of cysteine 128 are especially important. They can be switched on and off by light, and are therefore called step-function rhodopsins (3). Recent photocycle models have been proposed on the basis of visual and vibrational spectroscopy (4, 5), however, details of the photoreactions are still widely unknown. Here we present spectroscopical data on the slow cycling mutant C128T, with 200 fold extended lifetime of the conducting state. During the photocycle, a fraction of the proteins branches off into a sideway consisting of blue-shifted species which accumulate during prolonged illumination and play an important role in dark state regeneration (5). We will show that - depending on the illumination conditions - several structurally different dark states are regenerated. However, although such large structural alterations occur during the photocycle, they are not necessarily connected with on- and off-switching of the channel. A new model that connects chromophore isomerization and structural alterations of the protein will be discussed.

(1) Nagel, G. et al. (2002) Science 296, 2395-8.
(2) Nagel, G. et al. (2003) Proceedings of the National Academy of Sciences of the United States of America 100, 13940-5.